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(54) **DIAGNOSIS METHOD AND DIAGNOSIS KIT FOR DERMATOMYOSITIS**

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(57) **ABSTRACT**

An object of the present invention is to identify an antigen corresponding to anti-CADM-140 antibody, produce a recombinant protein, and establish an assay system by ELISA or the like. The present invention provides a kit for diagnosing dermatomyositis containing an MDA5 protein shown in SEQ ID NO: 4 or a fragment thereof that is recognized by anti-CADM-140 antibody.

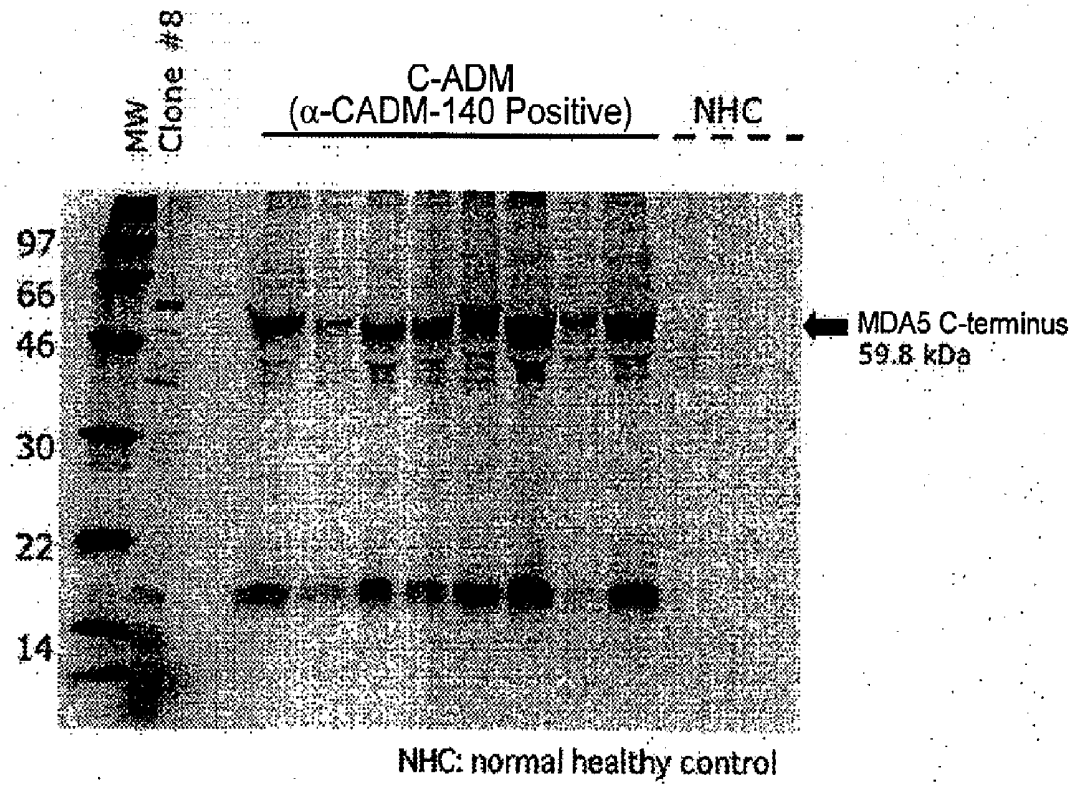


Fig. 1

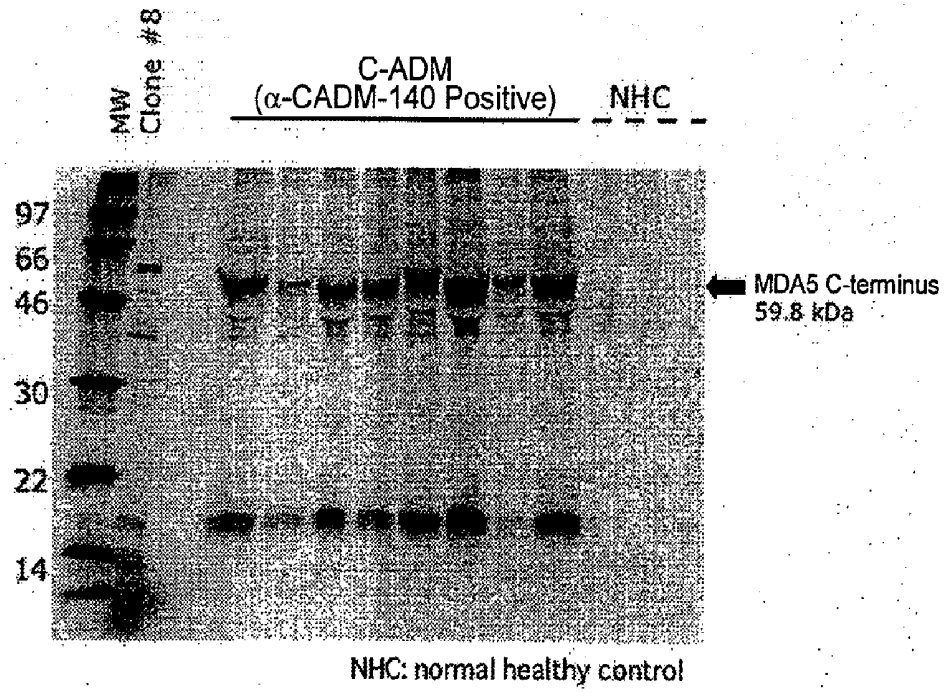

MW
Clone #8
C-ADM
(α-CADM-140 Positive)
NHC
97
66
46
30
22
14
MDA5 C-terminus
59.8 kDa
NHC: normal healthy control

Fig. 2

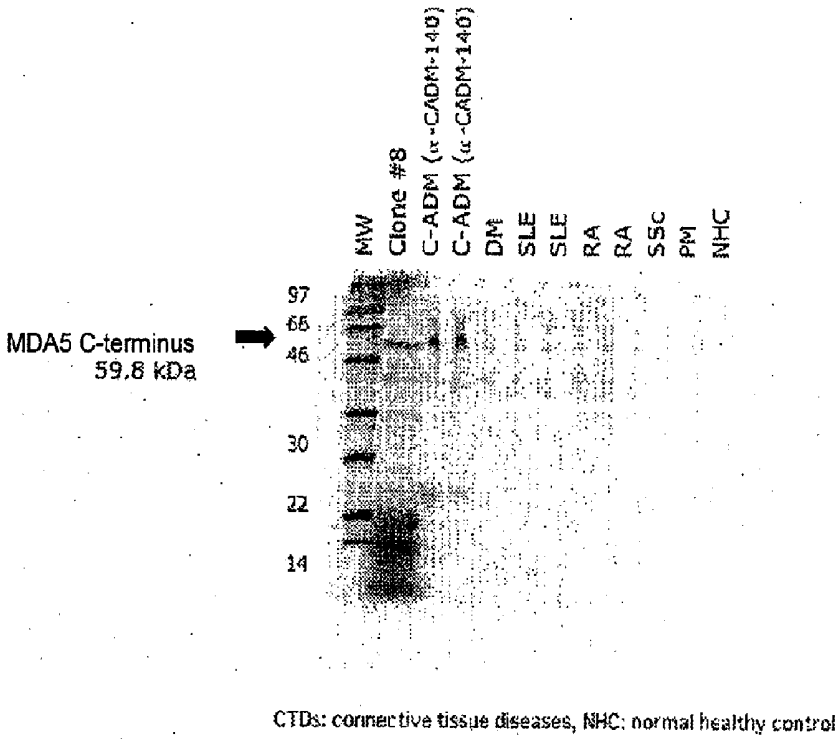

MW
Clone #8
C-ADM (α-CADM-140)
C-ADM (α-CADM-140)
DM
SLE
SLE
RA
RA
SSc
PM
NHC
97
66
46
30
22
14
MDA5 C-terminus
59.8 kDa
CTDs: connective tissue diseases, NHC: normal healthy control

Fig. 3

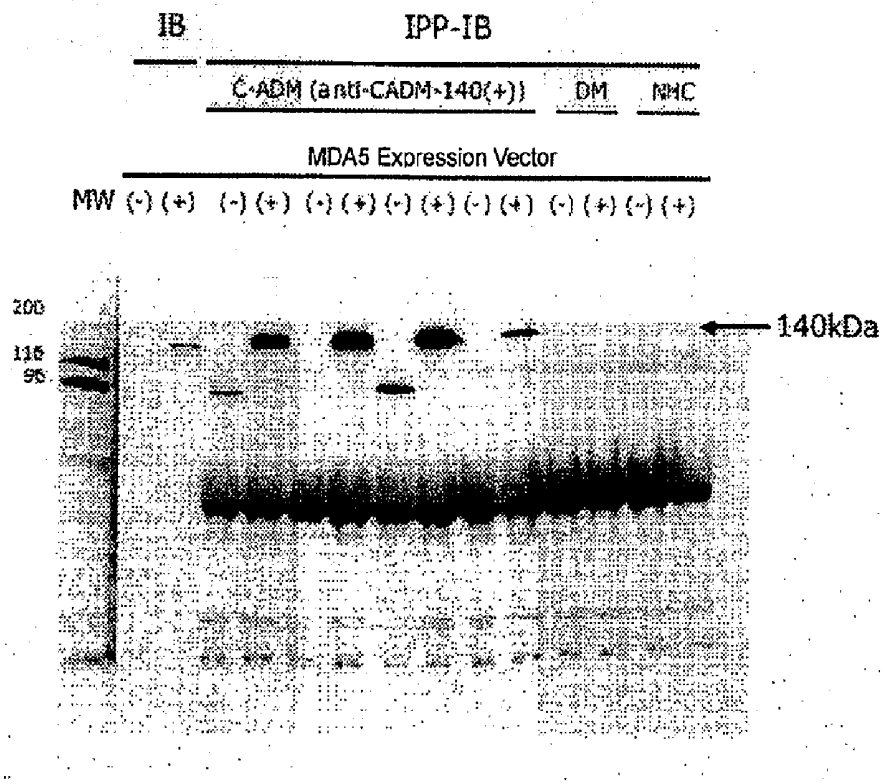

IB
IPP-IB
C-ADM (anti-CADM-140(+))
DM
NHC
MDA5 Expression Vector
MW (-) (+) (-) (+) (-) (+) (-) (+) (-) (+) (-) (+) (-) (+)
200
116
95
140kDa

Fig. 4

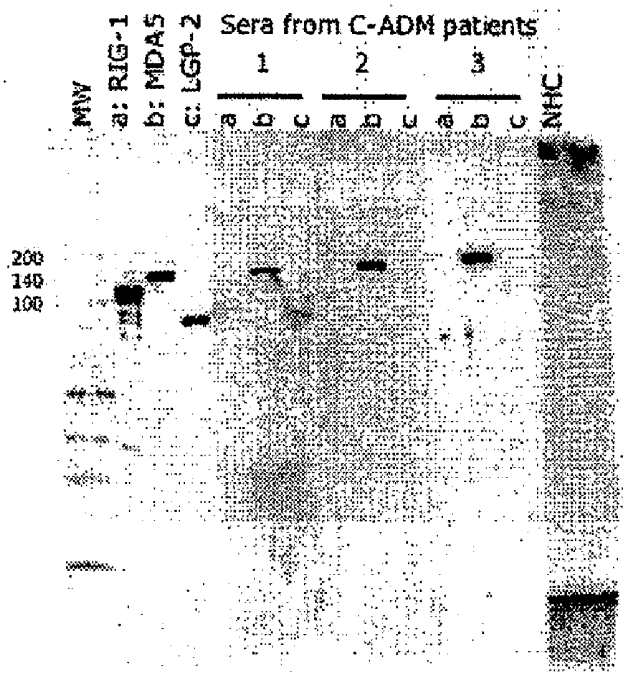
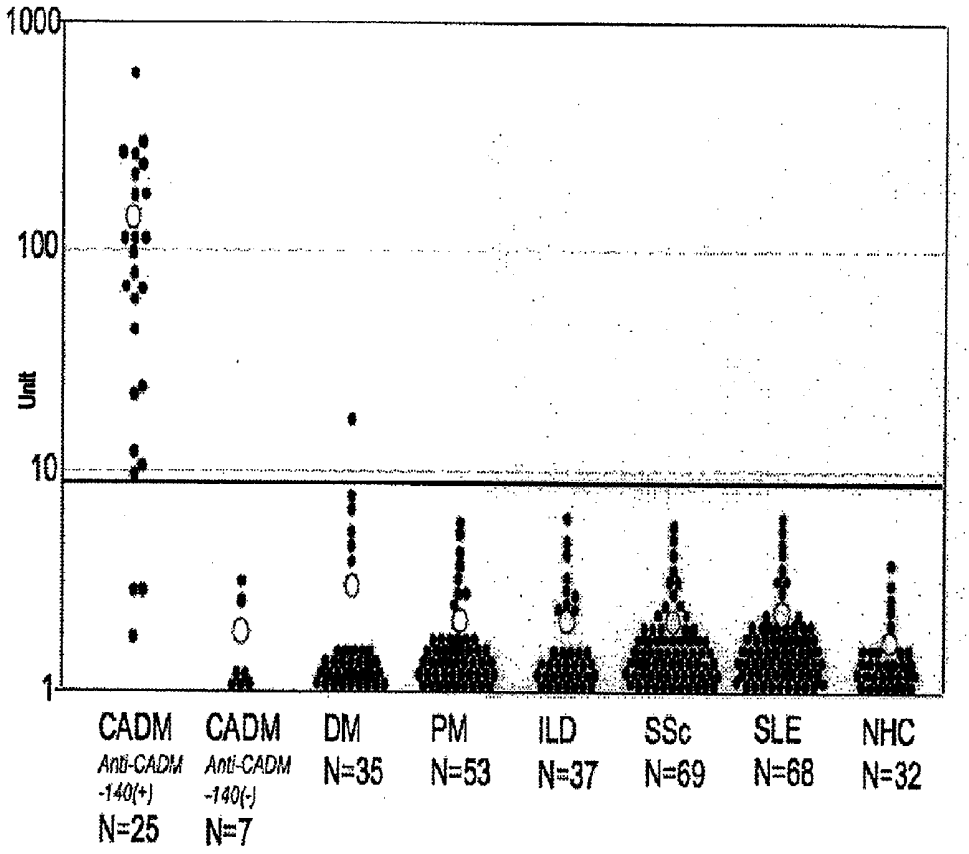

Sera from C-ADM patients
MW
a: RIG-1
b: MDA5
c: LGP-2
1
2
3
a b c a b c a b c
NHC
200
140
100

Fig. 5


1000
100
10
1
Unit
CADM
Anti-CADM
-140(+)
N=25
CADM
Anti-CADM
-140(-)
N=7
DM
N=35
PM
N=53
ILD
N=37
SSc
N=69
SLE
N=68
NHC
N=32

DIAGNOSIS METHOD AND DIAGNOSIS KIT FOR DERMATOMYOSITIS

TECHNICAL FIELD

[0001] The present invention relates to a method and a kit for diagnosing dermatomyositis, and use of an antigenic protein for diagnosing dermatomyositis.

BACKGROUND ART

[0002] A basic characteristic of collagen disease that causes it to be regarded as an autoimmune disease is the production of autoantibodies against various cellular components. It is known that many of the antigens corresponding to such autoantibodies are enzymes and regulatory factors that are essential to life processes. Investigating the molecular structures of the autoantibodies and antigens corresponding thereto (autoantigens), and their biological functions is expected to contribute to the elucidation of the pathogenesis of connective tissue diseases.

[0003] Polymyositis/dermatomyositis (PM/DM) is an inflammatory myopathy in which proximal muscle weakness and muscular pain caused by skeletal muscle inflammation predominate. Particularly, when typical skin symptoms such as heliotrope rash and Gottron's papules are observed, the patient is diagnosed with DM. Polymyositis/dermatomyositis is one of the autoimmune diseases, and various autoantibodies are known to develop. The presence of antibodies in the sera of PM/DM patients has been reported; such antibodies include anti-aminoacyl tRNA synthetase antibody (anti-ARS antibody), anti-SRP antibody, anti-Mi-2 antibody, and like antibodies specifically detected in myositis patients, as well as anti-U1RNP antibody, anti-SS-A antibody, and like autoantibodies associated with myositis. It is known that patients who are positive for the same myositis-specific autoantibody exhibit similar clinical characteristics; this is clinically useful for diagnosis, classification of disease types, selection of appropriate treatment methods, and estimation of prognosis.

[0004] In contrast, autoantibody negativity was considered to be one of the characteristics of clinically amyopathic DM (C-ADM), which is a sub-type of PM/DM. The presence of autoantibodies specific to C-ADM was unknown. It was known that C-ADM is resistant to clinical treatment and associated with poor prognostic rapidly progressive interstitial lung disease (RP-ILD). To save the life of C-ADM patients with RP-ILD, the efficacy of potent treatment from an early stage using a combination of high dose steroid therapy and immunosuppressive drug administration has been reported and recommended. For this reason, early diagnosis of C-ADM with associated RP-ILD is clinically important, and the establishment of new indicators that are useful for early diagnosis has been desired.

[0005] In light of the above background, the present inventors investigated the sera of normal healthy controls, those of patients with connective tissue diseases including C-ADM, and those of patients with idiopathic pulmonary fibrosis, according to an immunoprecipitation (IPP) method. The inventors found that a new autoantibody capable of recognizing a 140-kDa protein is present in the sera of C-ADM patients, and termed the new autoantibody "anti-CADM-140 antibody" (Non-Patent Literature 1 (NPL 1)).

[0006] The anti-CADM-140 antibody was not detected in any of the patients with connective tissue diseases other than C-ADM, patients with idiopathic pulmonary fibrosis (IPF), and normal healthy controls. Clinically, significantly frequent development of RP-ILD in anti-CADM-140 antibody-positive patients was observed, which suggests a correlation between the anti-CADM-140 antibody and RP-ILD (Non-Patent Document 2).

[0007] This phenomenon is considered to be useful for early diagnosis and treatment selection for extremely poor prognostic C-ADM with associated RP-ILD, and is thus expected to improve its prognosis.

[0008] MDA5 has a SNP (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=64135). The amino acid sequence and the base sequence thereof are available under NCBI Accession Number NM_022168. Patent Literature 1 (PTL 1) discloses the relationship between MDA5 and cancer.

[0009] The present inventors published Non-Patent Literature 3 (NPL 3), which discloses a technique to which the present invention pertains.

CITATION LIST

[0010] Patent Literature

[0011] PTL 1: Japanese Unexamined Patent Document No. 2003-531581

[0012] Non-Patent Literature

[0013] NPL 1: Arthritis Rheum. 46(9): S398, 2003

[0014] NPL 2: Arthritis Rheum. 52(5): 1571-1576, 2005

[0015] NPL 3: Arthritis Rheum. 60(7): 2193-2200, 2009

SUMMARY OF INVENTION

Technical Problem

[0016] Anti-CADM-140 antibody has been generally measured according to an IPP method using S^{35}-labeled leukemia-derived K562 cell extracts or HeLa cells. Although this is a reliable measurement method with high sensitivity and high specificity, the method utilizes isotopes and requires complicated procedures. Accordingly, the measurement method has been used only in a limited number of laboratories.

[0017] To apply anti-CADM-140 antibody measurement to actual clinical diagnosis, it is necessary to establish a measurement system that enables easy measurement of large quantities of samples. Identification of an antigen corresponding to anti-CADM-140 antibody, preparation of a recombinant protein, and establishment of a measurement system by ELISA etc., are important to establish such a system.

Solution to Problem

[0018] The present inventors used a HeLa cell cDNA library to clone an antigen gene corresponding to anti-CADM-140 antibody, and investigated the molecular sequence of the corresponding antigen protein. As a result, the inventors found that MDA5 (Melanoma Differentiation Associated Gene 5) is an antigen corresponding to anti-CADM-140 antibody and have accomplished the present invention based on this finding.

[0019] The present invention provides kits and methods for diagnosing dermatomyositis as itemized below.

Item 1. A kit for diagnosing dermatomyositis, comprising an MDA5 protein shown in SEQ ID NO: 4, or a fragment thereof that is recognized by anti-CADM-140 antibody.

Item 2. The kit for diagnosing dermatomyositis according to Item 1, comprising a C-terminal fragment of the MDA5 pro-

tein shown in SEQ ID NO: 2, or a fragment thereof that is recognized by anti-CADM-140 antibody.

Item 3. The kit according to Item 1, wherein the dermatomyositis is a clinically amyopathic dermatomyositis (C-ADM) associated with a high risk for developing rapidly progressive interstitial lung disease.

Item 4. The kit according to Item 1 used for measurement by enzyme-linked immunosorbent assay (ELISA).

[0020] Item 5. The kit according to Item 1, comprising:

(i) an antigen comprising an MDA5 protein or an immunogenic peptide thereof;

(ii) a medium suitable for use in a reaction between a sample of a subject and the antigen (i);

(iii) a reagent for detecting a complex of the antigen (i) and anti-CADM-140 antibody; and optionally

(iv) a reference sample not containing anti-CADM-140 antibody.

Item 6. A method for diagnosing dermatomyositis, comprising allowing a sample of a subject to react with an MDA5 protein or a fragment thereof that is recognized by anti-CADM-140 antibody; and diagnosing the subject as having dermatomyositis when the anti-CADM-140 antibody is detected in the sample.

Item 7. The method according to Item 5 wherein the sample is a blood, serum, or plasma sample.

Item 8. The method according to Item 6 wherein the anti-CADM-140 antibody in the sample is detected by ELISA.

Item 9. The method according to Item 6 comprising the following steps:

(i) depositing a specific amount of a peptide composition of the present invention on a plurality of wells of a microtiter plate;

(ii) diluting a sample of a subject suspected of having dermatomyositis, and adding the diluted sample to the wells;

(iii) washing the microtiter plate after incubation;

(iv) introducing a labeled anti-human immunoglobulin antibody to the wells of the microtiter plate; and

(v) detecting the amount of label bound to the antibody by comparison with a control.

Item 10. Use of an MDA5 protein or a fragment thereof that is recognized by anti-CADM-140 antibody to diagnose dermatomyositis.

ADVANTAGEOUS EFFECTS OF INVENTION

[0021] According to the present invention, extremely poor prognostic clinically amyopathic dermatomyositis associated with a high risk for developing extremely poor prognostic rapidly progressive interstitial lung disease can be detected with high accuracy.

[0022] The present invention enables quick measurement of anti-CADM-140 antibody in many samples. This is useful for early diagnosis and treatment selection for C-ADM with associated RP-ILD, and is expected to improve the prognosis of this disease whose treatment method has not been established and which has been considered as an extremely poor prognostic disease. Furthermore, the accumulation of anti-CADM-140 antibody-positive samples will enable the analysis of foreign antigens, such as a virus that is the target of a specific autoantibody, and contribute to the elucidation of the pathogenesis of C-ADM with associated RP-ILD.

BRIEF DESCRIPTION OF DRAWINGS

[0023] FIG. **1** shows the results of investigation of a reaction between a protein of Clone #8 (MDA5) as an antigen and the sera of C-ADM patients according to an immunoprecipitation method, which was performed after purifying the protein of Clone #8 (MDA5) and confirming the expression of the protein using an in vitro transcription/translation system. The Clone #8 (MDA5) reacted with all of the anti-CADM-140 antibody-positive serum samples, but it did not react with the sera of normal healthy controls.

[0024] FIG. **2** shows the results of investigation of a reaction between a recombinant protein of Clone #8 (MDA5) as an antigen, and the sera of anti-CADM-140 antibody-positive C-ADM patients, those of patients with other connective tissue diseases, and those of normal healthy controls, according to an immunoprecipitation method, which was performed after purifying the protein of Clone #8 (MDA5) and confirming expression of the protein using an in vitro transcription/translation system. The recombinant protein did not react with the sera of normal healthy controls or the sera of patients other than anti-CADM-140 antibody-positive C-ADM patients.

[0025] FIG. **3** shows the IPP-IB results. When anti-CADM-140 antibody was reacted with an MDA5-expressing African green monkey renal cell-derived COS7 cell extract, the resulting 140-kDa immunoprecipitate reacted with goat anti-MDA5 antibody.

[0026] FIG. **4** shows the immunoblotting results of the sera of anti-CADM-140 antibody-positive patients and those of normal healthy controls, obtained by using recombinant RIG-I, recombinant MDA5, and recombinant LGP-2 as antigens. The sera of anti-CADM-140 antibody-positive patients reacted only with the recombinant MDA5, whereas the sera of normal healthy controls (NHC) did not react at all.

[0027] FIG. **5** shows the ELISA results of the sera of anti-CADM-140 antibody-positive C-ADM patients, those of anti-CADM-140 antibody-negative C-ADM patients, those of PM/DM patients, those of scleroderma (SSc) patients, those of systemic lupus erythematosus (SLE) patients, those of ILD patients, and those of normal healthy controls, obtained by using MDA5 as an antigen substrate. The cutoff value is indicated by a horizontal line. The analytical sensitivity and analytical specificity of this ELISA system were 85% and 100%, respectively.

DESCRIPTION OF EMBODIMENTS

[0028] A feature of the present invention is an antigen (MDA5) specifically recognized by anti-CADM-140 antibody, which was found by the present inventor. MDA5 is one type of RIG-I family protein. RIG-I family proteins participate in a specific immune response that includes interferon type I production. RIG-I family proteins are highly homologous and contain a DexD/H-box helicase domain. In addition to MDA5, RIG-I and LPG-2 are also known as RIG-I family proteins. These proteins are very similar in sequence and structure, and are thus likely to be cross-reactive. Accordingly, the reactivity between anti-CADM-140 antibody and RIG-I family proteins was also investigated. The binding of anti-CADM-140 antibody to MDA5 depends on the conformation of the antigen, and anti-CADM-140 antibody prefer-

entially reacts with an MDA5 partially modified from its native structure. These factors made it difficult to determine that anti-CADM-140 antibody is an antibody to MDA5.

[0029] To identify anti-CADM-140 antibody, the present inventors purified an antigen protein corresponding thereto and tried to identify the antigen protein by mass spectrometry. However, it was impossible to purify a sufficient amount of the antigen protein, and identification was difficult. This was considered to be attributable to low amounts of antigen in the serum or to the affinity of the antigen-antibody reaction.

[0030] Further, in the early stages of C-ADM, it is difficult to distinguish the disease from other types of dermatomyositis, because C-ADM is difficult to diagnose and requires follow-up to carefully determine whether muscular symptoms are present or not.

[0031] However, the present inventors identified MDA5 specifically recognized by anti-CADM-140 antibody. As a result, it became clear that the clinical sensitivity and clinical specificity of the ELISA assay of dermatomyositis (C-ADM) are 69% and 99.6%, respectively. Although anti-CADM-140 antibody-negative C-ADM patients were previously difficult to diagnose or the diagnosis often took much time, the present invention enables quick diagnosis.

[0032] In this specification, "dermatomyositis" includes what is referred to as DM and clinically amyopathic dermatomyositis (C-ADM), which is a subtype of DM, but does not include polymyositis PM. Thus, the present invention provides a kit and a method for diagnosing whether the subject has DM or C-ADM or other diseases. DM is an inflammatory myopathy in which characteristic skin symptoms such as heliotrope rash and Gottron's papules, and proximal muscle weakness and/or muscular pain caused by skeletal muscle inflammation predominate. DM is generally diagnosed according to the diagnostic criteria of Bohan & Peter (Bohan A, Peter J B. Polymyositis and dermatomyositis. N Engl J Med 1975; 292: 344). However, C-ADM, which refers to the case in which cutaneous lesions characteristic of DM are exhibited but muscle weakness is not exhibited, does not meet the criteria of Bohan & Peter, and has been undiagnosable. In 2002, Sontheimer proposed new classification criteria in which a case of typical skin lesions without muscle weakness is called "C-ADM" (clinically amyopathic dermatomyositis), and "C-ADM" is classified as a subtype of DM [Sontheimer R D: Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis sine myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? J Am Acad Dermatol. 2002; 46: 626-36]. The target DM to be diagnosed according to the present invention includes those that meet the diagnostic criteria of Bohan & Peter or meet the classification criteria of Sontheimer.

[0033] The diagnostic kit and the diagnostic method of the present invention use an MDA5 protein or a fragment thereof recognized by anti-CADM-140 antibody to detect anti-CADM-140 antibody (hereinafter the "fragment recognized by anti-CADM-140 antibody" is sometimes simply referred to as an "immunogenic peptide"). Since the C-terminal 523-amino-acid sequence fragment (SEQ ID NO: 2) of MDA5 protein shown in SEQ ID NO: 4 binds to anti-CADM-140 antibody, an epitope recognized by anti-CADM-140 antibody is present in the C-terminal region of MDA5 protein. Insofar as this epitope is maintained, the MDA5 protein or the immunogenic peptide thereof may be a modified MDA5 protein or immunogenic peptide thereof that has one or more amino acid deletions, substitutions, additions, or insertions in a non-epitopic region thereof. The epitope typically consists of about 5 to about 10 amino acids, and particularly about 5 to about 8 amino acids. For example, the epitope of MDA5 can be determined by synthesizing a specific number of peptides (for example, 10 peptides at a time) while shifting by a fixed number of amino acids (for example, 5 amino acids) from the N-terminus of the C-terminal fragment of MDA5 shown in SEQ ID NO: 2 (for example, prepare a peptide consisting of the first to tenth amino acids, a peptide consisting of the sixth to 15th amino acids, a peptide consisting of the 11th to 20th amino acids, a peptide consisting of the 16th to 25th amino acids, . . .), and then investigating the reactivity of each of the peptide fragments with anti-CADM-140 antibody. When the epitope is determined or the location of the epitope is narrowed down, the MDA5 protein (immunogenic peptide) fragment recognized by anti-CADM-140 antibody can be shortened. A shorter immunogenic peptide is preferable from the viewpoint of ease of production. A peptide consisting of 40 or less amino acids, 30 or less amino acids, or 20 or less amino acids, particularly about 5 to about 10 amino acids, is preferable. RNA helicase is encoded by MDA5.

[0034] MDA5 is known to have an SNP. The MDA5 protein or the immunogenic peptide that is used in the present invention may have any type of SNP. SEQ ID NO: 3 represents a base sequence of MDA5. SEQ ID NO: 1 represents a base sequence encoding a C-terminal fragment of the MDA5 fragment shown in SEQ ID NO: 2. However, the MDA5 of other amino acid sequences/base sequences that are related by SNP, or alleles of MDA5 are also included in the scope of "MDA5" in this specification.

[0035] The MDA5 or the immunogenic peptide of the present invention can be expressed as a recombinant protein and produced by genetic engineering, or can be produced by chemical synthesis. Particularly, when the immunogenic peptide has a short sequence consisting of preferably 50 or less amino acids, more preferably 30 or less, even more preferably 20 or less, and particularly, 5 to 10 amino acids, chemical synthesis (a solid or liquid phase synthesis) is preferably used to produce the peptide. The MDA5 or the immunogenic peptide thereof may be used in the form of a monomer, and may be crosslinked by a polyfunctional crosslinking agent such as glutaraldehyde, or can be produced by genetic engineering by using DNA encoding a peptide in which immunogenic peptides are connected in tandem.

[0036] The anti-CADM-140 antibody may be obtained from any sample such as the blood, serum, plasma, cerebrospinal fluid, and lymph of a subject. Blood, serum, and plasma are preferable, and serum is particularly preferable as the sample.

[0037] The kit for diagnosing dermatomyositis of the present invention contains an MDA5 protein or an immunogenic peptide thereof. The kit may contain a substance for detecting an antigen-antibody complex of an MDA5 protein or an immunogenic peptide thereof, and anti-CADM-140 antibody. Examples of such substances include anti-human immunoglobulin antibodies that are labeled with peroxidases such as horseradish peroxidase (HRP), enzyme labels such as β-galactosidase, alkaline phosphatase, and luciferase, fluorescent labels such as FITC (fluorescein isothiocyanate) and RITC (tetramethylrhodamin isothiocyanate), or any other suitable labels, and particularly anti-human IgG antibodies labeled therewith.

[0038] Immunoassay is preferably used for the diagnostic kit and the diagnostic method of the present invention. Examples of the immunoassay include enzyme immunoassay (EIA), ELISA, fluoroimmunoassay (FIA), chemiluminescent immunoassay, immunoblotting (IB), Western blotting, immunostaining, and like methods.

[0039] The MDA5 protein or the immunogenic peptide used in the present invention is preferably bonded to a solid phase. Examples of such solid phases include agarose, wells of a microtiter plate, latex particles, and the like. Specific examples of the ELISA method include competitive immunoassay, sandwich immunoassay, and the like.

[0040] According to the method for diagnosing dermatomyositis of the present invention, a sample obtained from a subject is brought into contact with an MDA5 protein or an immunogenic peptide thereof to detect the presence or absence of an antigen-antibody complex of MDA5 or an immunogenic peptide thereof and anti-CADM-140 antibody by a physical or chemical method.

[0041] The method for diagnosing dermatomyositis of the present invention comprises, for example, the following steps:

(i) depositing a specific amount of the peptide composition of the present invention on a plurality of wells of a microtiter plate;

(ii) diluting a blood sample (serum or plasma) of a subject suspected of having dermatomyositis, and adding the diluted sample to the wells;

(iii) washing the microtiter plate after incubation;

(iv) adding human immunoglobulin (e.g., IgG) labeled with an enzyme label, a fluorescent label, or the like, to the wells of the microtiter plate; and

(v) detecting the amount of label bound to human immunoglobulin (the amount of substrate acted upon by an enzyme in the case of human immunoglobulin labeled with an enzyme label), compared to a control.

[0042] In a preferred embodiment, the diagnostic kit of the present invention may contain:

(i) an antigen comprising an MDA5 protein or an immunogenic peptide thereof (the antigen may be adhered to wells of a microtiter plate etc., and may further be blocked with a blocking agent such as skim milk, or an albumin such as bovine serum albumin (BSA) or I);

(ii) a medium suitable for the reaction between a sample of a subject and the antigen (i) (for example, buffers such as PBS);

(iii) a reagent for detecting a complex of the antigen (i) and anti-CADM-140 antibody (for example, a labeled anti-human immunoglobulin antibody bound to anti-CADM-140 antibody); and optionally

(iv) a reference sample not containing anti-CADM-140 antibody.

[0043] The "reference sample not containing anti-CADM-140 antibody" includes, for example, blood, serum, and plasma samples of normal healthy controls.

[0044] When the anti-human immunoglobulin antibody is labeled with peroxidase, anti-CADM-140 antibody can be detected by a process comprising adding a color development substrate such as tetramethylbenzidine; stopping the reaction with H_2SO_4; and measuring absorbance (450 nm: OD450) using a plate reader.

[0045] By testing many samples using the diagnostic kit of the present invention and comparing the obtained results with other clinical observations, more accurate criteria for determining whether a subject has DM, based on the measured amount of anti-CADM-140 antibody can be established.

EXAMPLES

[0046] The present invention is described below in more detail.

Example 1

Method for Early Diagnosis of C-ADM

[0047] An antigenic protein was expressed in *E. coli* by using an *E. coli* (XL1-Blue MRF) expressing lambda ZAP phage prepared by using a HeLa cell cDNA library. The expressed protein was transferred to a nitrocellulose membrane, and reacted with anti-CADM-140 antibody-positive serum to isolate positive clones. Next, the reactivity of the obtained positive clones with 10 serum samples of anti-CADM-140 antibody-positive C-ADM patients, 14 serum samples of anti-CADM-140 antibody-negative persons (two C-ADM samples, two PM samples, one sample each of systemic lupus erythematosus, scleroderma, and interstitial lung disease, and seven samples of normal healthy controls) was investigated. Among the 9 clones that were obtained, Clone #8 reacted with anti-CADM-140 antibody-positive serum samples at high frequency, i.e. 9 out of 10 anti-CADM-140 antibody-positive C-ADM serum samples. After the protein was purified and expression was confirmed using an in vitro transcription/translation assay, the reactivity of the sera of C-ADM patients with the protein as an antigen was investigated by immunoprecipitation. Clone #8 reacted with all of the anti-CADM-140 antibody-positive serum samples, but did not react with the sera of normal healthy controls (FIG. **1**). Further, Clone #8 did not react with the sera of patients with connective tissue diseases other than anti-CADM-140 antibody-positive C-ADM (FIG. **2**). Further, to determine the base sequence of Clone #8, plasmid DNA was excised from phage DNA from which the clone was obtained. The plasmid DNA was purified to determine the base sequence. Finally, a homology search was performed for the obtained base sequence, and the sequence was perfectly matched to the C-terminal sequence of MDA5.

[0048] An anti-CADM-140 antibody-positive serum was reacted with an MDA5-expressing African green monkey renal cell-derived COST cell extract by the IPP-IB method. The reactivity of 140 kDa of the obtained immunoprecipitate with goat anti-MDA5 antibody was investigated. When MDA5 was expressed, the immunoprecipitate reacted with goat anti-MDA5 antibody (FIG. **3**). Further, the reactivity of anti-CADM-140 antibody-positive sera and the sera of normal healthy controls with recombinant purified proteins of RIG-I family RIG-I, MD5, and LGP-2 as antigens was investigated. The sera of anti-CADM-140 antibody-positive patients reacted only with recombinant MDA5, whereas the sera of normal healthy controls did not react with recombinant MDA5 (FIG. **4**). It became clear from the above results that the antigen corresponding to anti-CADM-140 antibody is MDA5.

[0049] Further, the present inventors coated a 96-well plate with purified recombinant MDA5 as an antigen to establish an ELISA for an anti-ACDM-140 antibody assay. The diagnostic sensitivity and diagnostic specificity of the anti-CADM-140 antibody assay method were investigated.

[0050] More specifically, recombinant MDA5 was immobilized on a 96-well ELISA plate in an amount of 0.05 μg/well

and blocked with 3% BSA. Serum samples (each 250-fold diluted) were dispensed to the plate in an amount of 100 μl per well, and allowed to react at room temperature for 2 hours. As a secondary antibody, 5000-fold diluted peroxidase conjugated goat anti-human-IgG was used. Tetramethylbenzidine (1 mg/ml) was added as a color development substrate, and the reaction was stopped with H_2SO_4. The absorbance was measured with a plate reader (450 nm: OD_{450}). Using this assay system, the serum samples of C-ADM-containing collagen disease patients, those of interstitial lung disease (ILD) patients, and those of normal healthy controls were measured. The results showed that the reactivity of the sera of the anti-CADM-140 antibody-positive PM/DM patients was higher than that of the anti-CADM-140 antibody-negative PM/DM patients, scleroderma (SSc) patients, systemic lupus erythematosus (SLE) patients, ILD patients, and normal healthy controls (FIG. **5**). When the average+up to 10 times the standard derivation of normal healthy controls was defined as the normal cutoff range, the analytical sensitivity and the analytical specificity measured by this ELISA assay system were 85% and 100%, respectively. Thus, the results clearly show that this assay is an anti-CADM-140 antibody detection method with excellent sensitivity and specificity.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 4

<210> SEQ ID NO 1
<211> LENGTH: 1706
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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gaaaaccttg atcaactgaa aaaccaaata caggagccat gcaagaagtt tgccattgca 120

gatgcaacca gagaagatcc atttaaagag aaacttctag aaataatgac aaggattcaa 180

acttattgtc aaatgagtcc aatgtcagat tttggaactc aaccctatga acaatgggcc 240

attcaaatgg aaaaaaaagc tgcaaaagaa ggaaatcgca aagaacgtgt ttgtgcagaa 300

catttgagga agtacaatga ggccctacaa attaatgaca caattcgaat gatagatgcg 360

tatactcatc ttgaaacttt ctataatgaa gagaaagata agaagtttgc agtcatagaa 420

gatgatagtg atgagggtgg tgatgatgag tattgtgatg gtgatgaaga tgaggatgat 480

ttaaagaaac ctttgaaact ggatgaaaca gatagatttc tcatgacttt atttttgaa 540

aacaataaaa tgttgaaaag gctggctgaa aacccagaat atgaaaatga aaagctgacc 600

aaattaagaa ataccataat ggagcaatat actaggactg aggaatcagc acgaggaata 660

atctttacaa aaacacgaca gagtgcatat gcgctttccc agtggattac tgaaaatgaa 720

aaatttgctg aagtaggagt caaagcccac catctgattg gagctggaca cagcagtgag 780

ttcaaaccca tgacacagaa tgaacaaaaa gaagtcatta gtaaatttcg cactggaaaa 840

ataaatctgc ttatcgctac cacagtggca gaagaaggtc tggatattaa agaatgtaac 900

attgttatcc gttatggtct cgtcaccaat gaaatagcca tggtccaggc ccgtggtcga 960

gccagagctg atgagagcac ctacgtcctg gttgctcaca gtggttcagg agttatcgaa 1020

catgagacag ttaatgattt ccgagagaag atgatgtata aagctataca ttgtgttcaa 1080

aatatgaaac cagaggagta tgctcataag attttggaat tacagatgca aagtataatg 1140

gaaaagaaaa tgaaaaccaa gagaaatatt gccaagcatt acaagaataa cccatcacta 1200

ataactttcc tttgcaaaaa ctgcagtgtg ctagcctgtt ctggggaaga tatccatgta 1260

attgagaaaa tgcatcacgt caatatgacc ccagaattca aggaacttta cattgtaaga 1320

gaaaacaaag cactgcaaaa gaagtgtgcc gactatcaaa taaatggtga aatcatctgc 1380

aaatgtggcc aggcttgggg aacaatgatg gtgcacaaag gcttagattt gccttgtctc 1440

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aaaataagga attttgtagt ggttttcaaa aataattcaa caaagaaaca atacaaaaag   1500

tgggtagaat tacctatcac atttcccaat cttgactatt cagaatgctg tttatttagt   1560

gatgaggatt agcacttgat tgaagattct tttaaaatac tatcagttaa acatttaata   1620

tgattatgat taatgtattc attatgctac agaactgaca taagaatcaa taaaatgatt   1680

gttttactct gcaaaaaaaa aaaaaa                                       1706
```

<210> SEQ ID NO 2
<211> LENGTH: 523
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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1               5                  10                  15

Lys Thr Val Lys Glu Asn Leu Asp Gln Leu Lys Asn Gln Ile Gln Glu
            20                  25                  30

Pro Cys Lys Lys Phe Ala Ile Ala Asp Ala Thr Arg Glu Asp Pro Phe
        35                  40                  45

Lys Glu Lys Leu Leu Glu Ile Met Thr Arg Ile Gln Thr Tyr Cys Gln
    50                  55                  60

Met Ser Pro Met Ser Asp Phe Gly Thr Gln Pro Tyr Glu Gln Trp Ala
65                  70                  75                  80

Ile Gln Met Glu Lys Lys Ala Ala Lys Glu Gly Asn Arg Lys Glu Arg
                85                  90                  95

Val Cys Ala Glu His Leu Arg Lys Tyr Asn Glu Ala Leu Gln Ile Asn
            100                 105                 110

Asp Thr Ile Arg Met Ile Asp Ala Tyr Thr His Leu Glu Thr Phe Tyr
        115                 120                 125

Asn Glu Glu Lys Asp Lys Lys Phe Ala Val Ile Glu Asp Asp Ser Asp
    130                 135                 140

Glu Gly Gly Asp Asp Glu Tyr Cys Asp Gly Asp Glu Asp Glu Asp Asp
145                 150                 155                 160

Leu Lys Lys Pro Leu Lys Leu Asp Glu Thr Asp Arg Phe Leu Met Thr
                165                 170                 175

Leu Phe Phe Glu Asn Asn Lys Met Leu Lys Arg Leu Ala Glu Asn Pro
            180                 185                 190

Glu Tyr Glu Asn Glu Lys Leu Thr Lys Leu Arg Asn Thr Ile Met Glu
        195                 200                 205

Gln Tyr Thr Arg Thr Glu Glu Ser Ala Arg Gly Ile Ile Phe Thr Lys
    210                 215                 220

Thr Arg Gln Ser Ala Tyr Ala Leu Ser Gln Trp Ile Thr Glu Asn Glu
225                 230                 235                 240

Lys Phe Ala Glu Val Gly Val Lys Ala His His Leu Ile Gly Ala Gly
                245                 250                 255

His Ser Ser Glu Phe Lys Pro Met Thr Gln Asn Glu Gln Lys Glu Val
            260                 265                 270

Ile Ser Lys Phe Arg Thr Gly Lys Ile Asn Leu Leu Ile Ala Thr Thr
        275                 280                 285

Val Ala Glu Glu Gly Leu Asp Ile Lys Glu Cys Asn Ile Val Ile Arg
    290                 295                 300

Tyr Gly Leu Val Thr Asn Glu Ile Ala Met Val Gln Ala Arg Gly Arg
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305                 310                 315                 320

Ala Arg Ala Asp Glu Ser Thr Tyr Val Leu Val Ala His Ser Gly Ser
                325                 330                 335

Gly Val Ile Glu His Glu Thr Val Asn Asp Phe Arg Glu Lys Met Met
            340                 345                 350

Tyr Lys Ala Ile His Cys Val Gln Asn Met Lys Pro Glu Glu Tyr Ala
        355                 360                 365

His Lys Ile Leu Glu Leu Gln Met Gln Ser Ile Met Glu Lys Lys Met
    370                 375                 380

Lys Thr Lys Arg Asn Ile Ala Lys His Tyr Lys Asn Asn Pro Ser Leu
385                 390                 395                 400

Ile Thr Phe Leu Cys Lys Asn Cys Ser Val Leu Ala Cys Ser Gly Glu
                405                 410                 415

Asp Ile His Val Ile Glu Lys Met His His Val Asn Met Thr Pro Glu
            420                 425                 430

Phe Lys Glu Leu Tyr Ile Val Arg Glu Asn Lys Ala Leu Gln Lys Lys
        435                 440                 445

Cys Ala Asp Tyr Gln Ile Asn Gly Glu Ile Ile Cys Lys Cys Gly Gln
    450                 455                 460

Ala Trp Gly Thr Met Met Val His Lys Gly Leu Asp Leu Pro Cys Leu
465                 470                 475                 480

Lys Ile Arg Asn Phe Val Val Val Phe Lys Asn Asn Ser Thr Lys Lys
                485                 490                 495

Gln Tyr Lys Lys Trp Val Glu Leu Pro Ile Thr Phe Pro Asn Leu Asp
            500                 505                 510

Tyr Ser Glu Cys Cys Leu Phe Ser Asp Glu Asp
        515                 520


<210> SEQ ID NO 3
<211> LENGTH: 3434
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

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cggcctgaga gccctgtgga caacctcgtc attgtcaggc acagagcggt agaccctgct    120

tctctaagtg ggcagcggac agcggcacgc acatttcacc tgtcccgcag acaacagcac    180

catctgcttg ggagaaccct ctcccttctc tgagaaagaa agatgtcgaa tgggtattcc    240

acagacgaga atttccgcta tctcatctcg tgcttcaggg ccagggtgaa aatgtacatc    300

caggtggagc ctgtgctgga ctacctgacc tttctgcctg cagaggtgaa ggagcagatt    360

cagaggacag tcgccacctc cgggaacatg caggcagttg aactgctgct gagcaccttg    420

gagaagggag tctggcacct tggttggact cgggaattcg tggaggccct ccggagaacc    480

ggcagccctc tggccgcccg ctacatgaac cctgagctca cggacttgcc ctctccatcg    540

tttgagaacg ctcatgatga atatctccaa ctgctgaacc tccttcagcc cactctggtg    600

gacaagcttc tagttagaga cgtcttggat aagtgcatgg aggaggaact gttgacaatt    660

gaagacagaa accggattgc tgctgcagaa aacaatggaa atgaatcagg tgtaagagag    720

ctactaaaaa ggattgtgca gaaagaaaac tggttctctg catttctgaa tgttcttcgt    780

caaacaggaa acaatgaact tgtccaagag ttaacaggct ctgattgctc agaaagcaat    840
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gcagagattg agaatttatc acaagttgat ggtcctcaag tggaagagca acttctttca 900

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tcatcttttg cagattcttc tgtagtttca gaatcagaca caagtttggc agaaggaagt 1020

gtcagctgct tagatgaaag tcttggacat aacagcaaca tgggcagtga ttcaggcacc 1080

atgggaagtg attcagatga agagaatgtg gcagcaagag catccccgga gccagaactc 1140

cagctcaggc cttaccaaat ggaagttgcc cagccagcct tggaagggaa gaatatcatc 1200

atctgcctcc ctacagggag tggaaaaacc agagtggctg tttacattgc caaggatcac 1260

ttagacaaga agaaaaaagc atctgagcct ggaaaagtta tagttcttgt caataaggta 1320

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ggagaagatg ctggtgttca attgtcagac ttttccctca ttatcattga tgaatgtcat 1560

cacaccaaca aagaagcagt gtataataac atcatgaggc attatttgat gcagaagttg 1620

aaaaacaata gactcaagaa agaaaacaaa ccagtgattc cccttcctca gatactggga 1680

ctaacagctt cacctggtgt tggaggggcc acgaagcaag ccaaagctga agaacacatt 1740

ttaaaactat gtgccaatct tgatgcattt actattaaaa ctgttaaaga aaaccttgat 1800

caactgaaaa accaaatata ggagccatgc aagaagtttg ccattgcaga tgcaaccaga 1860

gaagatccat ttaaagagaa acttctagaa ataatgacaa ggattcaaac ttattgtcaa 1920

atgagtccaa tgtcagattt tggaactcaa ccctatgaac aatgggccat tcaaatggaa 1980

aaaaaagctg caaaagaagg aaatcgcaaa gaacgtgttt gtgcagaaca tttgaggaag 2040

tacaatgagg ccctacaaat taatgacaca attcgaatga tagatgcgta tactcatctt 2100

gaaactttct ataatgaaga gaaagataag aagtttgcag tcatagaaga tgatagtgat 2160

gagggtggtg atgatgagta ttgtgatggt gatgaagatg aggatgattt aaagaaacct 2220

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acacgacaga gtgcatatgc gctttcccag tggattactg aaaatgaaaa atttgctgaa 2460

gtaggagtca aagcccacca tctgattgga gctggacaca gcagtgagtt caaacccatg 2520

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aatgatttcc gagagaagat gatgtataaa gctatacatt gtgttcaaaa tatgaaacca 2820

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tgcaaaaact gcagtgtgct agcctgttct ggggaagata tccatgtaat tgagaaaatg 3000

catcacgtca atatgacccc agaattcaag gaactttaca ttgtaagaga aaacaaagca 3060

ctgcaaaaga agtgtgccga ctatcaaata aatggtgaaa tcatctgcaa atgtggccag 3120

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cctatcacat ttcccaatct tgactattca gaatgctgtt tatttagtga tgaggattag 3300

cacttgattg aagattcttt taaaatacta tcagttaaac atttaatatg attatgatta 3360

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aaaaaaaaaa aaaa 3434

<210> SEQ ID NO 4
<211> LENGTH: 1025
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Cys Phe Arg Ala Arg Val Lys Met Tyr Ile Gln Val Glu Pro Val Leu
20 25 30

Asp Tyr Leu Thr Phe Leu Pro Ala Glu Val Lys Glu Gln Ile Gln Arg
35 40 45

Thr Val Ala Thr Ser Gly Asn Met Gln Ala Val Glu Leu Leu Leu Ser
50 55 60

Thr Leu Glu Lys Gly Val Trp His Leu Gly Trp Thr Arg Glu Phe Val
65 70 75 80

Glu Ala Leu Arg Arg Thr Gly Ser Pro Leu Ala Ala Arg Tyr Met Asn
85 90 95

Pro Glu Leu Thr Asp Leu Pro Ser Pro Ser Phe Glu Asn Ala His Asp
100 105 110

Glu Tyr Leu Gln Leu Leu Asn Leu Leu Gln Pro Thr Leu Val Asp Lys
115 120 125

Leu Leu Val Arg Asp Val Leu Asp Lys Cys Met Glu Glu Glu Leu Leu
130 135 140

Thr Ile Glu Asp Arg Asn Arg Ile Ala Ala Ala Glu Asn Asn Gly Asn
145 150 155 160

Glu Ser Gly Val Arg Glu Leu Leu Lys Arg Ile Val Gln Lys Glu Asn
165 170 175

Trp Phe Ser Ala Phe Leu Asn Val Leu Arg Gln Thr Gly Asn Asn Glu
180 185 190

Leu Val Gln Glu Leu Thr Gly Ser Asp Cys Ser Glu Ser Asn Ala Glu
195 200 205

Ile Glu Asn Leu Ser Gln Val Asp Gly Pro Gln Val Glu Glu Gln Leu
210 215 220

Leu Ser Thr Thr Val Gln Pro Asn Leu Glu Lys Glu Val Trp Gly Met
225 230 235 240

Glu Asn Asn Ser Ser Glu Ser Ser Phe Ala Asp Ser Ser Val Val Ser
245 250 255

Glu Ser Asp Thr Ser Leu Ala Glu Gly Ser Val Ser Cys Leu Asp Glu
260 265 270

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Ser Leu Gly His Asn Ser Asn Met Gly Ser Asp Ser Gly Thr Met Gly
        275                 280                 285

Ser Asp Ser Asp Glu Glu Asn Val Ala Ala Arg Ala Ser Pro Glu Pro
    290                 295                 300

Glu Leu Gln Leu Arg Pro Tyr Gln Met Glu Val Ala Gln Pro Ala Leu
305                 310                 315                 320

Glu Gly Lys Asn Ile Ile Ile Cys Leu Pro Thr Gly Ser Gly Lys Thr
                325                 330                 335

Arg Val Ala Val Tyr Ile Ala Lys Asp His Leu Asp Lys Lys Lys Lys
            340                 345                 350

Ala Ser Glu Pro Gly Lys Val Ile Val Leu Val Asn Lys Val Leu Leu
        355                 360                 365

Val Glu Gln Leu Phe Arg Lys Glu Phe Gln Pro Phe Leu Lys Lys Trp
    370                 375                 380

Tyr Arg Val Ile Gly Leu Ser Gly Asp Thr Gln Leu Lys Ile Ser Phe
385                 390                 395                 400

Pro Glu Val Val Lys Ser Cys Asp Ile Ile Ile Ser Thr Ala Gln Ile
                405                 410                 415

Leu Glu Asn Ser Leu Leu Asn Leu Glu Asn Gly Glu Asp Ala Gly Val
            420                 425                 430

Gln Leu Ser Asp Phe Ser Leu Ile Ile Ile Asp Glu Cys His His Thr
        435                 440                 445

Asn Lys Glu Ala Val Tyr Asn Asn Ile Met Arg His Tyr Leu Met Gln
    450                 455                 460

Lys Leu Lys Asn Asn Arg Leu Lys Lys Glu Asn Lys Pro Val Ile Pro
465                 470                 475                 480

Leu Pro Gln Ile Leu Gly Leu Thr Ala Ser Pro Gly Val Gly Gly Ala
                485                 490                 495

Thr Lys Gln Ala Lys Ala Glu Glu His Ile Leu Lys Leu Cys Ala Asn
            500                 505                 510

Leu Asp Ala Phe Thr Ile Lys Thr Val Lys Glu Asn Leu Asp Gln Leu
        515                 520                 525

Lys Asn Gln Ile Gln Glu Pro Cys Lys Lys Phe Ala Ile Ala Asp Ala
    530                 535                 540

Thr Arg Glu Asp Pro Phe Lys Glu Lys Leu Leu Glu Ile Met Thr Arg
545                 550                 555                 560

Ile Gln Thr Tyr Cys Gln Met Ser Pro Met Ser Asp Phe Gly Thr Gln
                565                 570                 575

Pro Tyr Glu Gln Trp Ala Ile Gln Met Glu Lys Lys Ala Ala Lys Glu
            580                 585                 590

Gly Asn Arg Lys Glu Arg Val Cys Ala Glu His Leu Arg Lys Tyr Asn
        595                 600                 605

Glu Ala Leu Gln Ile Asn Asp Thr Ile Arg Met Ile Asp Ala Tyr Thr
    610                 615                 620

His Leu Glu Thr Phe Tyr Asn Glu Glu Lys Asp Lys Lys Phe Ala Val
625                 630                 635                 640
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Ile Glu Asp Asp Ser Asp Glu Gly Gly Asp Asp Glu Tyr Cys Asp Gly
                645                 650                 655

Asp Glu Asp Glu Asp Asp Leu Lys Lys Pro Leu Lys Leu Asp Glu Thr
            660                 665                 670

Asp Arg Phe Leu Met Thr Leu Phe Phe Glu Asn Asn Lys Met Leu Lys
        675                 680                 685

Arg Leu Ala Glu Asn Pro Glu Tyr Glu Asn Glu Lys Leu Thr Lys Leu
    690                 695                 700

Arg Asn Thr Ile Met Glu Gln Tyr Thr Arg Thr Glu Glu Ser Ala Arg
705                 710                 715                 720

Gly Ile Ile Phe Thr Lys Thr Arg Gln Ser Ala Tyr Ala Leu Ser Gln
                725                 730                 735

Trp Ile Thr Glu Asn Glu Lys Phe Ala Glu Val Gly Val Lys Ala His
            740                 745                 750

His Leu Ile Gly Ala Gly His Ser Ser Glu Phe Lys Pro Met Thr Gln
        755                 760                 765

Asn Glu Gln Lys Glu Val Ile Ser Lys Phe Arg Thr Gly Lys Ile Asn
    770                 775                 780

Leu Leu Ile Ala Thr Thr Val Ala Glu Glu Gly Leu Asp Ile Lys Glu
785                 790                 795                 800

Cys Asn Ile Val Ile Arg Tyr Gly Leu Val Thr Asn Glu Ile Ala Met
                805                 810                 815

Val Gln Ala Arg Gly Arg Ala Arg Ala Asp Glu Ser Thr Tyr Val Leu
            820                 825                 830

Val Ala His Ser Gly Ser Gly Val Ile Glu His Glu Thr Val Asn Asp
        835                 840                 845

Phe Arg Glu Lys Met Met Tyr Lys Ala Ile His Cys Val Gln Asn Met
    850                 855                 860

Lys Pro Glu Glu Tyr Ala His Lys Ile Leu Glu Leu Gln Met Gln Ser
865                 870                 875                 880

Ile Met Glu Lys Lys Met Lys Thr Lys Arg Asn Ile Ala Lys His Tyr
                885                 890                 895

Lys Asn Asn Pro Ser Leu Ile Thr Phe Leu Cys Lys Asn Cys Ser Val
            900                 905                 910

Leu Ala Cys Ser Gly Glu Asp Ile His Val Ile Glu Lys Met His His
        915                 920                 925

Val Asn Met Thr Pro Glu Phe Lys Glu Leu Tyr Ile Val Arg Glu Asn
    930                 935                 940

Lys Ala Leu Gln Lys Lys Cys Ala Asp Tyr Gln Ile Asn Gly Glu Ile
945                 950                 955                 960

Ile Cys Lys Cys Gly Gln Ala Trp Gly Thr Met Met Val His Lys Gly
                965                 970                 975

Leu Asp Leu Pro Cys Leu Lys Ile Arg Asn Phe Val Val Val Phe Lys
            980                 985                 990

Asn Asn Ser Thr Lys Lys Gln Tyr  Lys Lys Trp Val Glu  Leu Pro Ile
        995                 1000                1005

Thr Phe  Pro Asn Leu Asp Tyr  Ser Glu Cys Cys Leu  Phe Ser Asp
    1010                1015                1020

Glu Asp
    1025
```

1. A kit for diagnosing dermatomyositis, comprising an MDA5 protein shown in SEQ ID NO: 4, or a fragment thereof that is recognized by anti-CADM-140 antibody.

2. The kit for diagnosing dermatomyositis according to claim **1**, comprising a C-terminal fragment of the MDA5 protein shown in SEQ ID NO: 2, or a fragment thereof that is recognized by anti-CADM-140 antibody.

3. The kit according to claim **1**, wherein the dermatomyositis is a clinically amyopathic dermatomyositis (C-ADM) associated with a high risk for developing rapidly progressive interstitial lung disease.

4. The kit according to claim **1** used for measurement by enzyme-linked immunosorbent assay (ELISA).

5. The kit according to claim **1**, comprising:

(i) an antigen comprising an MDA5 protein or an immunogenic peptide thereof;

(ii) a medium suitable for use in a reaction between a sample of a subject and the antigen (i);

(iii) a reagent for detecting a complex of the antigen (i) and anti-CADM-140 antibody and; and optionally

(iv) a reference sample not containing anti-CADM-140 antibody.

6. A method for diagnosing dermatomyositis, comprising allowing a sample of a subject to react with an MDA5 protein or a fragment thereof that is recognized by anti-CADM-140 antibody; and diagnosing the subject as having dermatomyositis when the anti-CADM-140 antibody is detected in the sample.

7. The method according to claim **6** wherein the sample is a blood, serum, or plasma sample.

8. The method according to claim **6** wherein the anti-CADM-140 antibody in the sample is detected by ELISA.

9. The method according to claim **6** comprising the following steps:

(i) depositing a specific amount of a peptide composition of the present invention on a plurality of wells of a microtiter plate;

(ii) diluting a sample of a subject suspected of having dermatomyositis, and adding the diluted sample to the wells;

(iii) washing the microtiter plate after incubation;

(iv) introducing a labeled anti-human immunoglobulin antibody to the wells of the microtiter plate; and

(v) detecting the amount of label bound to the antibody by comparison with a control.

10. Use of an MDA5 protein or a fragment thereof that is recognized by anti-CADM-140 antibody to diagnose dermatomyositis.

* * * * *

专利名称(译)	皮肌炎的诊断方法和诊断试剂盒		
公开(公告)号	US20110165600A1	公开(公告)日	2011-07-07
申请号	US13/059444	申请日	2009-07-31
[标]申请(专利权)人(译)	正隆蚣 佐藤真司 藤田TAKASHI		
申请(专利权)人(译)	正隆蚣 佐藤真司 藤田TAKASHI		
当前申请(专利权)人(译)	正隆蚣 佐藤真司 藤田TAKASHI		
[标]发明人	KUWANA MASATAKA SATO SHINJI FUJITA TAKASHI		
发明人	KUWANA, MASATAKA SATO, SHINJI FUJITA, TAKASHI		
IPC分类号	G01N33/53 C12N9/00		
CPC分类号	G01N33/6854 G01N33/5743		
优先权	2008223789 2008-09-01 JP		
外部链接	Espacenet USPTO		

摘要(译)

本发明的目的是鉴定对应于抗CADM-140抗体的抗原，产生重组蛋白，并通过ELISA等建立测定系统。本发明提供了用于诊断皮肌炎的试剂盒，其含有SEQ ID NO：4中所示的MDA5蛋白或其抗CDM-140抗体识别的片段。